

Phusion PCR

1. Reaction Setup on ice
 1. 10 μ l HF or GC Buffer
 2. 1 μ l 10 mM dNTPs
 3. 2.5 μ l 10 μ M Forward Primer
 4. 2.5 μ l 10 μ M Reverse Primer
 5. 1 μ l Template á 20-30 ng/ μ l
 6. Optional: 1 μ l DMSO
 7. 0.5 μ l Phusion DNA Polymerase
 8. Fill with Nuclease-free water to 50 μ l
2. Thermocycling conditions
 1. 30 s 98°C Initial Denaturation
 2. max 20 Cycles for preparative, 35 for analytical purposes
 1. 10 s 98 °C
 2. Ta °C 30 s
 3. 72° C 30 s/kb
 3. Final Extension 72 °C 5 min